

CLAIMS

1/ A method for detecting cytochrome c in a given biological sample, comprising :

5 - adding to said sample an efficient amount of two redox couples allowing for a cycling oxido-reduction of cytochrome c, said couples comprising an oxidizing agent consisting of cytochrome c oxidase enzyme and a reducing agent specific for cytochrome c with a reduced co-factor;

10 - measuring, by a biophysical system depending on the co-factor and allowing to distinguish the co-factor oxidized form from the reduced form, the oxidation of the co-factor which is oxidized during said cycling redox reaction; the amount of the co-factor oxidized form being correlated to the concentration of cytochrome c in the sample.

2/ The method of claim 1, wherein said measurement is compared to measurements performed with standard cytochrome c.

3/ The method of claim 1 or 2, wherein the reducing agent is NADH-cytochrome c reductase or NADPH-cytochrome c reductase and the reduced co-factor is NADH or NADPH respectively.

4/ The method of claim 1 to 3, wherein the co-factor is detected by absorption spectrophotometry at 340 nm.

5/ The method of any of claims 1 to 4, wherein said agents are, for example but not limited to, under liquid, dried or lyophilised form and obtained by purification of recombinant or natural compounds or by chemical synthesis.

6/ The method of any of claims 1 to 5, optimized for any new screening protocol or adaptaded to any existing screening procedure.

7/ A kit for detecting cytochrome c in sample to be tested, comprising

- two redox couples for a cycling oxido-reduction of cytochrome c; said couples comprising an oxidizing agent

consisting of cytochrome c oxidase enzyme and a reducing agent specific for cytochrome c with a reduced co-factor.

8/ The kit of claim 7, wherein the reducing agent is NADH-cytochrome c reductase and the co-factor is NADH.

5 9/ The kit of claim 7, wherein the reducing agent is NADPH-cytochrome c reductase and the co-factor is NADPH.

10/ The kit of any of claims 7 to 9, further comprising cytochrome c as a reference standard.

11/ The kit of any of claims 7 to 10, further comprising a
10 buffer.

12/ The kit of claims 7 to 11, wherein said agents are, for example but not limited to, under liquid, dried or lyophilised form, and obtained by purification of recombinant or natural compounds or by chemical synthesis.

15 13/ The kit of claims 7 to 12, defined for laboratory research only.

14/ The kit of claims 7 to 12, defined for diagnostic use.

15/ The kit of any of claims 7 to 14, optimized for any format of container, for example but not limited to, 96-well
20 microplates, 384-well microplates, 1 mL cuvettes.

16/ The kit of any of claims 7 to 15, optimized for detecting cytochrome c in mitochondrial supernatants.

17/ The kit of any of claims 7 to 15, optimized for detecting cytochrome c in cytosol extracts.

18/ The kit of any of claims 7 to 15, optimized for detecting cytochrome c in any other biological sample expected to contain cytochrome c.

19/ The kit of claim 18, with reagents supplied for the preparation of mitochondrial and/or cytosolic fractions.

20/ The kit of claim 19, with methodology for the preparation of mitochondrial and/or cytosolic fractions.